

Evaluation of Fungal Pathogens as Biological Control Agents for Cogongrass (*Imperata cylindrica*)¹

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Abstract: Based on field surveys and evaluations in the greenhouse, two fungal pathogens, *Bipolaris sacchari* and *Drechslera gigantea*, were identified as promising biological control agents for cogongrass. In greenhouse trials, the application of spore suspensions of these fungi containing 10⁵ spores/ml in a 1% aqueous gelatin solution to cogongrass plants and their incubation in a dew chamber for 24 h resulted in disease symptoms that ranged from discrete lesions to complete blighting of leaves. Disease severity (DS), based on a rating scale for southern corn leaf blight with 50% as the maximum DS rating, ranged from 42 to 49%. In greenhouse experiments, the application of spores formulated in an oil emulsion composed of 4% horticultural oil, 10% light mineral oil, and 86% water resulted in higher levels of foliar blight with no dew exposure or shorter periods of dew exposure (4, 8, or 12 h) as compared with the application of spores formulated in 1% gelatin. Field trials demonstrated that under natural conditions, the application of a spore and an oil emulsion mixture containing 10⁵ spores/ml of either fungus could cause foliar injury from disease and phytotoxic damage from the oil emulsion. Depending on the application rate (100 or 200 ml/plot), the level of foliar injury ranged from 40 to 86% (based on a field assessment scale of 0 to 100% foliar injury) with *B. sacchari* as the test fungus. However, with *D. gigantea* as the test fungus, foliar injury ranged from 9 to 70% depending on the application volume and the oil concentration used. Although *B. sacchari* and *D. gigantea* were capable of causing foliar blight on cogongrass, the regenerative ability of the rhizomes allowed cogongrass to recover from the damage caused by these fungi. However, the level of injury caused by these fungi is sufficient to support their use as components for integrated management of cogongrass.

Nomenclature: Cogongrass, *Imperata cylindrica* (L.) Beauv. #3 IMPCY; corn, *Zea mays* L.

Additional index words: Bioherbicide, biological control, *Bipolaris sacchari* (E.J. Butler) Shoemaker, *Drechslera gigantea* (Heald & F.A. Wolf) Ito.

Abbreviations: CRD, completely randomized design; DAI, days after inoculation; DS, disease severity; RH, relative humidity; SOE, spore and oil emulsion mixture; WAI, weeks after inoculation; WM, weed mortality.

INTRODUCTION

Cogongrass is ranked as the seventh worst weed in the world and is the most serious perennial weed of southern and eastern Asia (Holm et al. 1977). It infests more than 500 million ha worldwide, including 200 million ha in Asia and Africa, and about 100,000 ha in the southeastern United States (Dickens 1974; Falvey 1981;

Holm et al. 1977). It is a weed of 35 economically important crops in 73 countries in the tropics (Holm et al. 1977). In the United States, it has been reported as a weed in Alabama, Georgia, Florida, Louisiana, Mississippi, South Carolina, Texas, and Virginia (Byrd and Bryson 1999). Presently, it occurs in Florida in natural and disturbed areas such as grasslands, roadsides, forests, recreational areas, and reclaimed mined areas (Coile and Shilling 1993). Cogongrass has the potential to be a more serious weed in the southeastern United States, particularly in agronomic crops grown with minimum tillage (Patterson et al. 1980).

The use of mechanical control methods to manage cogongrass is not justifiable on the basis of either cost or feasibility (Dozier et al. 1998; Shilling et al. 1995). Chemical control methods, aside from being costly, may

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³ Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

cause nontarget plant injury and select for herbicide-resistant plants. Biological control using plant pathogens and insects has been considered as a potential means to manage cogongrass infestations (Van Loan et al. 2002). A number of insects and pathogens have been found in association with cogongrass in many countries. Insects, such as the least skipper [*Ancyloxypha numitor* (Fabricius)], the fiery skipper [*Hylephila phyleus* (Drury)] (Bryson 1985, 1987), and the small branded swift [*Pelopidas mathias* (Fabricius)] (Masuzawa et al. 1983), reportedly feed on cogongrass, but these lepidopteran species were reported to be unsuitable as control agents because their larvae also feed on several desirable grass species (Bryson and Carter 1993). In Malaysia, several fungal plant pathogens have been evaluated as potential control agents for cogongrass, including *Colletotrichum caudatum* (Sacc.) Peck, *Colletotrichum graminicola* (Ces.) G.W. Wils., *Aschochyta* sp., *Puccinia rufipes* Diet., *Didymaria* sp., and *Dinesmasporium* sp. (Caunter 1996). In the United States, Chase et al. (1996) evaluated several fungal isolates from diseased cogongrass plants collected from various sites in Florida. Four of the six isolates tested were pathogenic to cogongrass and caused 60 to 100% disease incidence, but none caused mortality. Yandoc (2001) screened several indigenous fungi isolated from cogongrass and other grasses for their pathogenicity to cogongrass and determined that an isolate of *Bipolaris sacchari* (E.J. Butler) Shoemaker from cogongrass and *Drechslera gigantea* (Heald & Wolf) Ito from large crabgrass [*Digitaria sanguinalis* (L.) Scop.] had potential as biological control agents. The objectives of this study were to (1) compare the efficacy of the two fungi and their combination on cogongrass, (2) determine whether an oil formulation would increase their biocontrol efficacy, and (3) determine the efficacy of these fungi under field conditions and determine the effect of application rate and oil concentration on the efficacy of the fungi.

MATERIALS AND METHODS

General Procedures. Fresh cultures of *B. sacchari* and *D. gigantea* were started from stored cultures by growing them out on vegetable juice⁴ agar (200 ml juice, 800 ml water, 3 g CaCO₃, and 14 g agar⁵). Inoculum (spores) for the greenhouse and field experiments was produced by methods described previously by Yandoc (2001). Spore concentration, as number of spores per milliliter

of suspension, was determined with the aid of a hemacytometer.

Cogongrass rhizomes were collected from a cogongrass stand near Lake Alice, University of Florida campus, Gainesville, FL. The rhizomes were cut into 4-cm segments and planted in plastic trays (13 by 17 by 56 cm) that contained a commercial potting medium,⁶ and 4 g of a slow-release fertilizer⁷ was added. After 2 to 4 wk in a greenhouse, rhizomes with healthy shoots were transplanted into clay pots (11.5-cm diameter, 11-cm height) or plastic pots (15-cm diameter, 17.5-cm height).

The disease severity (DS) data from the greenhouse and the foliar injury data from field experiments were transformed using an arcsine square root transformation. ANOVA was performed using the SAS-General Linear Models⁸ procedure, and the means were compared using Duncan's multiple range test at the 5% level. Trials of the same experiments were analyzed separately when there was a significant treatment by trial interaction effect or when the variances between trials were not homogenous.

Greenhouse Experiments. The biological control efficacy of *B. sacchari*, *D. gigantea*, and their mixture was tested under greenhouse conditions. Spores of *B. sacchari* and *D. gigantea* were suspended in sterilized water with 1% unflavored gelatin⁹ as a sticker and humectant. Spore concentrations ranged from 10⁵ to 10⁶ spores/ml. Four-week-old cogongrass plants were inoculated with the spore suspension until incipient runoff. Inoculated and untreated control plants (sprayed with 1% gelatin alone) were incubated in a dew chamber for 24 h (27 ± 1 C, 100% relative humidity [RH]) and then transferred to a greenhouse (35/25 + 5 C and 85 + 5% RH, with 400 µE/s m² light level at midday) for further observations. The bioherbicidal efficacy of the isolates was measured in terms of DS or percent blighted leaf area. The DS levels were rated on a per-leaf basis with the aid of a pictorial key developed for the southern corn leaf blight, with 50% as the maximum disease level (James 1971). In this rating scale, the level of disease or foliar damage ranges from 0 to 50%, where 50% is the maximum rating and denotes that half the leaf area is necrotic. DS levels higher than 50% are not considered discernable to the naked eye; hence, the maximum was

⁴ V8 100% vegetable juice; Campbell Soup Company, Campbell Place, Camden, NJ 08103-1701.

⁵ Granulated agar; Fisher Scientific, 1 Reagent Lane, Fair Lawn, NJ 07410.

⁶ Metromix 300; Scott-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041.

⁷ Osmocote 14-14-14 controlled-release fertilizer; Scott-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041.

⁸ SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

⁹ Knox unflavored gelatin; Nabisco Inc., 100 DeForest Avenue, East Hanover, NJ 07936.

set at 50%. Disease assessments were done at 7 and 21 d after inoculation (DAI). The experiment had a completely randomized design (CRD) with three replications per treatment and was done thrice. Each replication was composed of one pot with three cogongrass plants.

The effect of formulating spores in an oil emulsion and exposure to no dew or 4, 8, 12, or 24 h of dew on the efficacy of *B. sacchari* and *D. gigantea* also were tested in the greenhouse. Spores of *B. sacchari* and *D. gigantea* were formulated with an oil emulsion composed of 8% horticultural oil,¹⁰ 20% light mineral oil,¹¹ and 72% sterile water. The oil emulsion was added to an equal volume of aqueous spore suspension (1×10^6 spores/ml), resulting in a formulation with a final oil concentration of 14% and half the original spore concentration. The control treatment was composed of spores formulated in 1% aqueous gelatin solution. The spore and gelatin mixture was prepared in a manner similar to that described above; however, a 2% gelatin solution was mixed with the spore suspension instead of the oil emulsion. A gelatin-only treatment was not included in the experiment because we have previously determined that gelatin had no phytotoxic effect on cogongrass. Oil emulsion-only treatment was excluded as well because an earlier study showed that oil plus spores can cause significantly higher foliar damage (100% weed mortality [WM]) than oil emulsion alone (30% WM) (C. B. Yandoc, unpublished data). Four-week-old cogongrass plants (three plants per pot, three pots per treatment) in clay pots (11.5-cm diameter, 11.0-cm height) or square plastic pots (8 by 8 by 9 cm) were inoculated with spores of *B. sacchari* or *D. gigantea* (5.0×10^5 spores/ml) formulated with 14% oil emulsion or 1% gelatin using hand-pumped sprayers until the foliage was completely wetted. Inoculated plants were randomly arranged and incubated in a dew chamber (100% RH, 28 ± 1 C) for 4, 8, 12, or 24 h or kept in the greenhouse immediately after inoculation (0 h dew). DS was assessed at 7 and 21 DAI. The experiment was conducted twice.

Field Trials. Field trials were conducted in 1999 to determine the biocontrol efficacy of *B. sacchari* and *D. gigantea* under field conditions and the effect of applying a spore and oil emulsion mixture (SOE) containing 5.0×10^5 spores/ml (*B. sacchari*) or 4.0×10^5 spores/ml (*D. gigantea*) formulated with 18% (8% horticultural

oil plus 10% light mineral oil) or 26% oil (16% horticultural oil and 10% light mineral oil) at the rate of 100 or 200 ml/plot. The concentration of the horticultural and light mineral oils in the SOE used in the greenhouse trials was modified for the field trials (8 and 16% horticultural oil instead of 4% and equal or double the concentration of light mineral oil) because field-grown cogongrass plants had tougher leaves than the greenhouse-grown plants. This emulsion formulation was used to reduce the likelihood of the SOE being washed off by heavy rain. The two oil concentrations also were tested to determine which concentration worked better in the field. Control treatments consisted of oil emulsion alone (18 or 26% applied at the rate of 100 or 200 ml/plot) and untreated checks.

Field trials were done at two locations in Florida; the trials done in Gainesville had a CRD, but the Waldo trial was done in a randomized complete block design because some plots were under a tree canopy, whereas others were in full sunlight. Experimental plots at either location measured 0.25 m², and there were four replications per treatment. Plots in Gainesville, which were under full sunlight, were inoculated late in the afternoon. Plots in Waldo were inoculated early in the morning.

The inoculated and control plants were observed for symptoms of disease and injury at the first and second weeks after inoculation (WAI). Disease assessments were done every week from the second WAI until the sixth WAI. Data were collected similarly for all field trials. Because the visual estimates were done on a per-plot basis, the rating scale for the greenhouse tests was not used. Instead, severity of foliar injury (combination of disease and phytotoxic damage from the oil emulsion) was estimated from the percentage of blighted above-ground biomass per plot (0 to 100%). The estimates were classified into severity classes based on the Barratt-Horsfall scale (Barratt and Horsfall 1945), and the corresponding mean class value was used instead of the actual visual estimates.

RESULTS AND DISCUSSION

Efficacy of *B. sacchari* and *D. gigantea* Isolates Tested Singly or in a Mixture. At the early stage of the disease caused by either fungus, the lesions were discrete, brown to dark reddish-brown, and elongate or spindle shaped with white or pale centers and had brown tapering ends. Lesion size ranged from 1.0 to 2.0 mm in length and approximately 0.5 mm in diameter. As the disease progressed, areas around some of the discrete lesions turned yellow. Lesions very close together appeared as one

¹⁰ Sunspray 6E; Sun Company Inc., Delaware Avenue and Grn, Marcus Hook, PA 19061-0835.

¹¹ Light mineral oil; Fisher Scientific, 100 Reagent Avenue, Fair Lawn, NJ 07410.

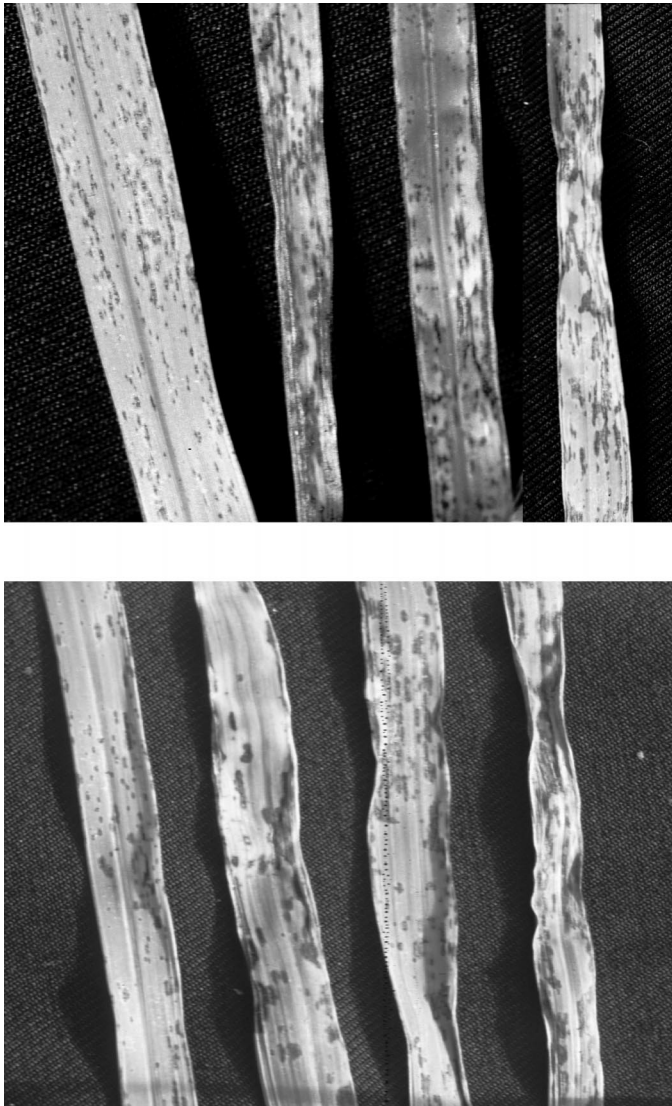


Figure 1. Disease symptoms caused by *Bipolaris sacchari* (top) and *Drechslera gigantea* (bottom) on cogongrass leaves from inoculations done in a greenhouse. The symptoms were observed at 7 d after inoculation.

large, irregularly shaped, necrotic area because of the yellow borders that eventually turned brown (Figure 1). The discrete lesions did not increase appreciably in size, but the yellowing of the borders became widespread and caused large leaf areas to become necrotic. Tips and edges of infected younger leaves turned dark green to brown and eventually became desiccated, and the leaves folded over. Older leaves exhibited more severe disease symptoms; large necrotic areas with yellow borders and subsequent blighting of most of the leaf blade, beginning at the tips and leaf edges, were observed within 72 h after inoculation.

No secondary disease spread was observed in the greenhouse. Both *B. sacchari* and *D. gigantea* caused

Table 1. Disease severity of cogongrass inoculated with *Bipolaris sacchari*, *Drechslera gigantea*, or the mixture of the two fungal isolates.^{a,b}

| Treatment | Disease severity ^c | | Weed mortality ^{c,d} |
|--------------------|-------------------------------|--------|-------------------------------|
| | 7 DAI | 21 DAI | 21 DAI |
| | % | | |
| Trial 1 | | | |
| Untreated control | 0 c | 0 d | 0 c |
| <i>B. sacchari</i> | 47 a | 43 b | 33 b |
| <i>D. gigantea</i> | 44 b | 35 c | 0 c |
| Mixture | 49 a | 48 a | 67 b |
| Trial 2 | | | |
| Untreated control | 0 c | 0 b | 0 b |
| <i>B. sacchari</i> | 46 ab | 41 a | 27 ab |
| <i>D. gigantea</i> | 44 b | 38 a | 27 ab |
| Mixture | 48 a | 42 a | 47 a |
| Trial 3 | | | |
| Untreated control | 0 b | 0 d | 0 b |
| <i>B. sacchari</i> | 46 a | 46 a | 39 a |
| <i>D. gigantea</i> | 45 b | 36 b | 13 b |
| Mixture | 42 a | 35 b | 0 b |

^a Abbreviation: DAI, days after inoculation.

^b All inoculations were done in the greenhouse. All inoculated plants were provided with 24 h of dew.

^c In each trial, within each DAI, figures followed by the same letter are not significantly different at $P = 0.05$, as determined by the Duncan's multiple range test.

^d Weed mortality percentage values were calculated from the average number of dead plants per pot.

severe foliar blight, but high disease levels (35 to 49% DS) did not always translate to high WM (0 to 67%) (Table 1). The infection was localized and limited to the sprayed foliage. Leaves that emerged after the plants were inoculated were free of disease, and the healthy regrowth decreased the proportion of diseased tissue per plant and, consequently, the DS rating after a few weeks. Regrowth indicated that the pathogens did not adversely affect the underground reproductive structures of the cogongrass and did not produce secondary disease cycles. The application of *B. sacchari*, *D. gigantea*, and their mixture caused comparable levels of disease at 7 and 21 DAI. The application of the mixture had no synergistic effect on the DS levels on cogongrass. Untreated plants showed no disease symptoms.

Efficacy of *B. sacchari* and *D. gigantea* Applied in an Oil Emulsion or Gelatin Under Different Dew Periods. In the first trial, the formulation (i.e., oil emulsion or gelatin), the length of dew exposure, and the interaction between the two significantly influenced the DS level caused by *B. sacchari*. Plants inoculated with spores applied in oil emulsion exhibited severe foliar blighting (48 to 50% DS) (Figure 2A) and death (89 to 100% WM) (data not shown) regardless of the length of dew exposure (0, 4, 8, 12, or 24 h). In the second trial,

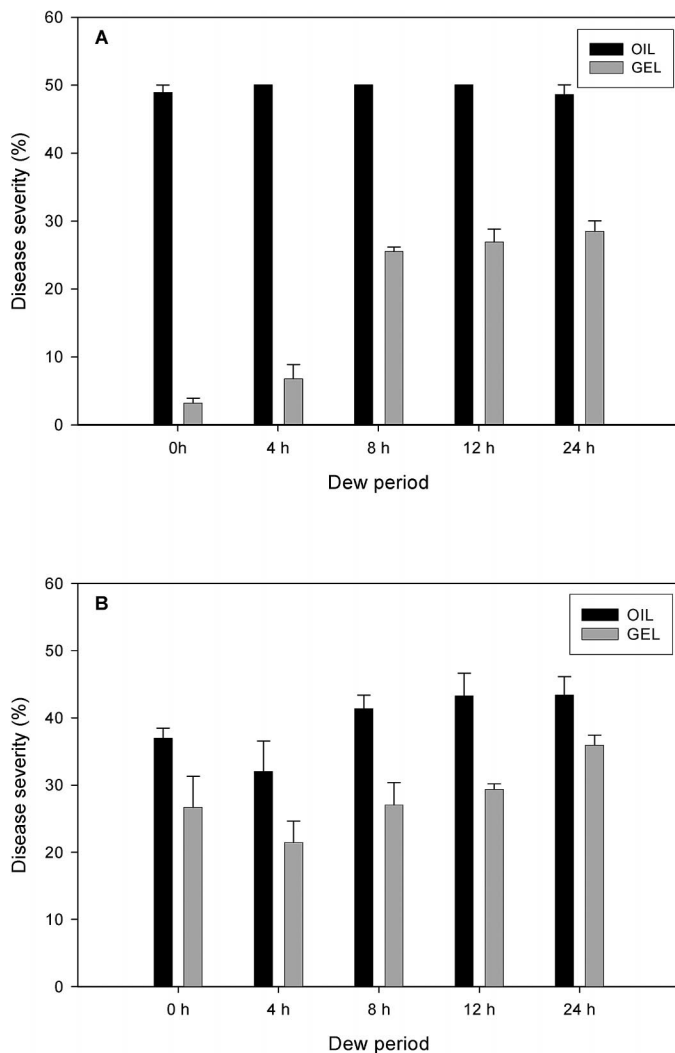


Figure 2. Comparison of foliar blight, as disease severity (% DS), caused by *Bipolaris sacchari* on cogongrass when spores were applied with an oil emulsion (OIL), composed of 14% horticultural oil plus 10% mineral oil, and 1% aqueous gelatin solution (GEL). Results from trial 1 (A) and trial 2 (B) are shown. Vertical bars denote the standard error of the means.

formulation and length of dew exposure affected DS levels, but there was no interaction effect. Spores applied in oil emulsion caused higher DS (32 to 43%) than spores formulated in gelatin (21 to 36% DS) over all dew periods tested (Figure 2B). High DS levels achieved with the oil formulation did not consistently result in high WM; mortality for the first trial was very high (89 to 100% across all dew periods) but in the second trial WM ranged from 11 to 33% over all dew periods tested (data not shown). Within 21 d, plants treated with spores in the gelatin formulation exhibited mostly distinct lesions along the leaf blade and only minor blighting of the leaf tips. Plants sprayed with SOE exhibited severe blighting or plant death.

In both trials with *D. gigantea* as the test fungus, there

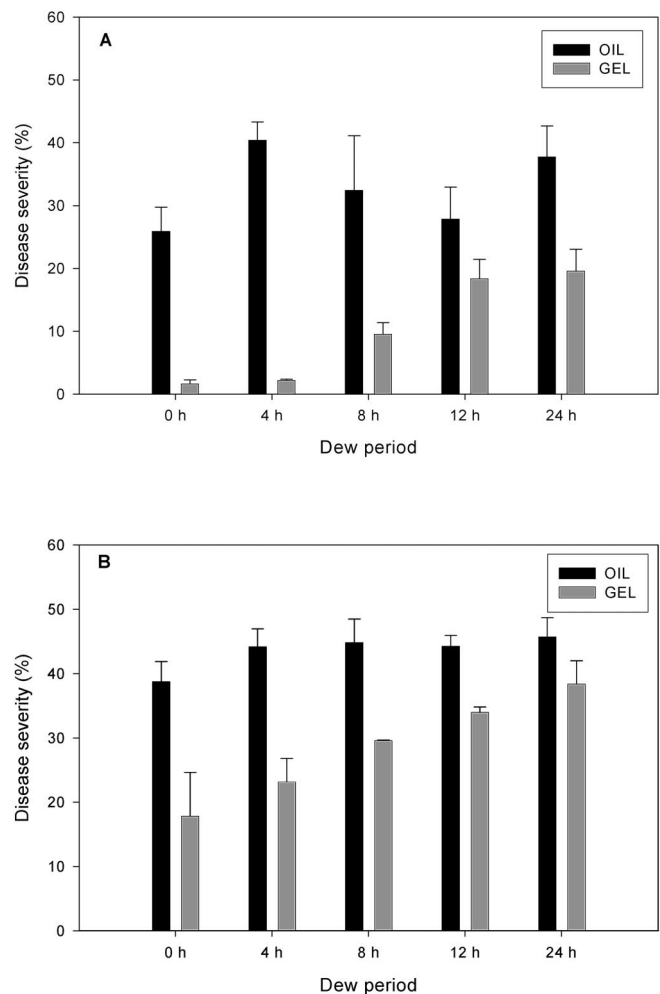


Figure 3. Comparison of foliar blight, as disease severity (% DS \pm standard error), caused by *Drechslera gigantea* on cogongrass when spores were applied with an oil emulsion (OIL), composed of 14% horticultural oil plus 10% mineral oil, and 1% aqueous gelatin solution (GEL). Results from trial 1 (A) and trial 2 (B) are shown. Vertical bars denote the standard error of the means.

was a significant effect of formulation and length of dew exposure on the DS level but there was no interaction effect between the two factors. The oil formulation caused significantly higher DS (26 to 40% in trial 1, 38 to 46% in trial 2) with 0 to 24 h of postinoculation dew exposure, as compared with spores formulated in 1% gelatin (2 to 20% DS in trial 1, 18 to 38% DS in trial 2) (Figures 3A and 3B). High DS levels achieved with the oil formulation did not translate to high WM; mortality ranged from 22 to 44% and 1 to 33% in the first and second trials, respectively (data not shown).

Under greenhouse conditions, foliar injury was more severe when the spores were applied with an oil emulsion, which acts as a spreader and a sticker. Severe foliar injury resulted from the larger areas of the foliage being covered with spores and the oil emulsion, which is

slightly phytotoxic. We hypothesize that the phytotoxicity of the oil emulsion caused damage on the leaf tissue and also allowed easier penetration by the spores, regardless of the duration of dew exposure; hence, this formulation is ideal for use in the field where dew periods may vary. However, the addition of oil only enhanced the level of DS and not WM. The inability of the infection to spread to the underground structures allowed the rhizomes to persist, allowing for regrowth despite the foliar damage.

Various additives have been used to improve or modify spore germination, pathogen stability and virulence, environmental requirements, or host range of bioherbicidal fungi (Boyette et al. 1996). Among the various additives tested are invert (water-in-oil) emulsions and oil (oil-in-water) emulsions. Oil emulsions are suitable bioherbicide carriers because of their surfactant properties, their ability to protect spores from exposure and drying, and their ability to retain some water (Auld 1993). Oil emulsions have been used in several bioherbicide formulations with variable success (Greaves et al. 1998). Under controlled environment experiments, the efficacy of some pathogens used for weed biocontrol was improved when the spores were formulated in oils (Auld 1993; Boyette 1994). Formulation in oil increased the efficacy of *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore (Boyette 1994) and reduced dew requirements of *Alternaria helianthii* (Hansf.) Tub. Et Nish. (Abbas and Egley 1996) and *Mycocentrospora acerina* (R. Hartig) Deighton (Potyka 1996). Chandramohan and Charudattan (2001) reported effective control of seven weedy grasses by formulating a combination of three pathogens in an oil emulsion composed of 40% horticultural oil and 10% paraffin oil. The oil emulsion formulation caused significantly higher disease levels as compared with a 0.5% aqueous psyllium mucilloid formulation (Chandramohan and Charudattan 2001).

Field Evaluation of *B. sacchari* and *D. gigantea* Biocontrol Efficacy. In both trials with *B. sacchari*, there was a significant treatment effect on the severity of foliar blighting or injury to cogongrass. The most severe damage (>80%) resulted from the application of 200 ml of the SOE with either 18 or 26% oil. Damage from the oil emulsion alone at either concentration was generally minor (<10% for trial 1 and <20% for trial 2) and significantly lower than the SOE treatments (Figure 4A). The experiment demonstrated that *B. sacchari* can cause severe foliar damage on cogongrass, especially when a larger volume of SOE (containing twice the number of spores and twice the volume of oil emulsion) was ap-

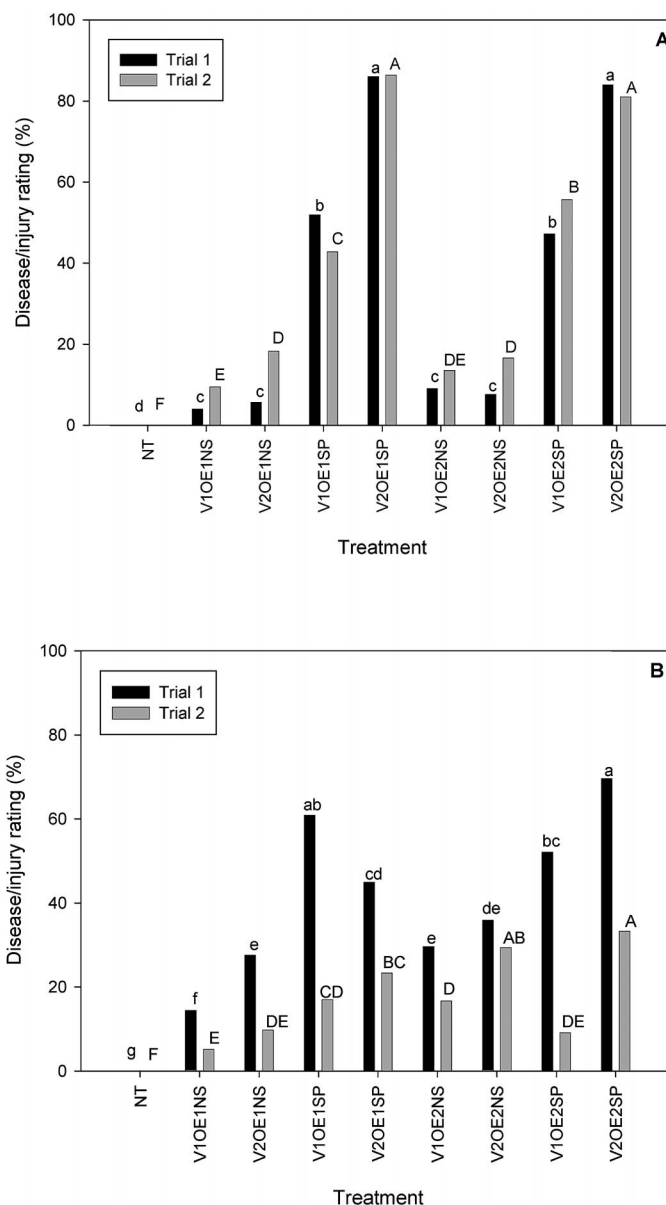


Figure 4. Biocontrol efficacy of *Bipolaris sacchari* (A) and *Drechslera gigantea* (B) formulated with oil emulsion under field conditions. Each bar represents an average foliar injury rating taken from 2 until 6 wk after inoculation. Treatments were: NT = untreated check; V1OE1NS = 100 ml of 18% oil emulsion; V2OE1NS = 200 ml of 26% oil emulsion; V1OE1SP = 100 ml of 18% oil plus spores; V2OE1SP = 200 ml of 18% oil plus spores; V1OE2NS = 100 ml of 26% oil emulsion; V2OE2NS = 200 ml of 26% oil emulsion; V1OE2SP = 100 ml of 26% oil plus spores; and V2OE2SP = 200 ml of 26% oil plus spores. Within a trial, bars with similar letters are not significantly different at $P = 0.05$, as determined by Duncan's multiple range test.

plied. It also was determined that increasing the amount of oil in the SOE from 18 to 26% did not necessarily increase the level of foliar blight or injury.

With *D. gigantea* as the test fungus, the most severe damage was only 70%, which was caused when the spores were applied with 26% oil at the rate of 200 ml/

plot (Figure 4B). The level of foliar blighting or injury caused by all the other SOE treatments in trial 1 ranged from 50 to 60%, whereas the treatments of oil emulsion alone caused 14 to 35% damage. In trial 2, the most severe level of damage was only 33%, which was caused by the application of 200 ml SOE containing 26% oil. The level of foliar blighting or injury caused by all other SOE treatments ranged from a mere 9 to 23%, whereas the oil emulsion-alone treatments caused 5 to 29% damage (Figure 3).

Despite the variability of results between trials, it was demonstrated that both fungi can cause disease on cogongrass under field conditions. However, the results from field experiments also showed that *D. gigantea* did not perform as well as *B. sacchari* even when similar inoculum concentrations were applied (5.0×10^5 spores/ml and 4.0×10^5 spores/ml) or when temperatures and RH during and immediately after inoculation were not very different. Temperature during the *D. gigantea* field inoculations ranged from 26 to 28 C and the RH ranged from 66 to 72%, whereas the temperature during *B. sacchari* inoculations ranged from 27 to 30 C and RH was 68% during both trials. The differences in the levels of foliar damage in the field trials may have been due to factors that affected the ability of the pathogens to infect the weed (quality of spores used in each trial or virulence of the fungus itself) or predisposed it to infection or injury, made it less susceptible, or allowed it to recover quickly from the disease or the phytotoxic effects of the oil emulsion. These factors may include the environmental conditions during the course of the experiments (temperature regimen and the amount of rainfall) and possibly the differences in the susceptibility of the cogongrass populations that were sprayed.

The results from the field trials indicated that application of a high volume of the SOE was necessary to achieve high levels of foliar blighting in the field. Because higher SOE contained twice the number of spores and twice the amount of carrier, the fungus and the oil covered more leaf area; hence, a greater amount of foliage was damaged. According to Shrum (1982), uniform distribution of inoculum on plant surface sufficiently early in the season is essential for creating epidemics. For a weed such as cogongrass, which can produce numerous leaves during a growing season and grow to a height of 3 m, a high application rate is needed to ensure complete coverage of the massive amount of foliage. Complete coverage of the leaf surfaces with inoculum also is important because *B. sacchari* and *D. gigantea* have not been observed to produce secondary infections on co-

gongrass; therefore, there will be no continuous source of inoculum. Hence, the level of cogongrass control in the field ultimately will depend on the level of initial infection and foliar blighting.

The requirement for large volumes of inoculum to assure biocontrol efficacy also has been demonstrated by other studies (Imaizumi et al. 1997; Klein and Auld 1995; Mortensen and Makowski 1989). Imaizumi et al. (1997) determined that there is a distinct dose-response effect of *Xanthomonas campestris* (Pammel) Dowson pv. *poae* (strain JT-P482) cell concentration and carrier (water) volume on the level of annual bluegrass (*Poa annua* L.) control. Klein and Auld (1995) reported that high water volumes favored development of disease caused by *Colletotrichum orbiculare* (Berk. & Mont.) Arx on spiny cocklebur (*Xanthium spinosum* L.). However, they also observed that when environmental conditions were conducive to disease development, the lower number of spores and lower carrier volumes were adequate. Mortensen and Makowski (1989) observed that the application of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. f.sp. *malvae* in a higher compared with a lower volume of water produced a more uniform initial infection on round-leaved mallow (*Malva pusilla* Sm.) in the field.

The production of new leaves or shoots by inoculated plants in the greenhouse, including those that were severely diseased, indicated that the infection caused by these fungi do not always kill plants because the regenerative potential of the rhizomes is not reduced. In the field, where plants have rhizomes greater in size and number than greenhouse-grown plants, it is expected that the potential for blighted plants to produce new shoots will be greater. However, we do not have rhizome biomass data from the field experiments to support this. Our study only demonstrated the level of foliar blighting that can be achieved using *B. sacchari* or *D. gigantea* and possible ways to improve the field efficacy of these fungi (by increasing the volume of the SOEs). As with any other method of weed control, the use of bioherbicides alone will not provide complete control of cogongrass, and it will require integration with other control methods.

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